

Recovery Plan

Cotton Leaf Curl Viral Disease Complex

Caused by Cotton leaf curl virus complex (Begomovirus, Geminiviridae): A group of whitefly-transmitted ssDNA viruses with ssDNA satellites, causing leaf curl disease of cotton, vegetables, and ornamentals

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Cultivated cotton, *Gossypium* species (L.), has been a major source of food, feed, and fiber, worldwide, for at least 7,000 years. Globally, about 32.6 million hectares are devoted to cotton cultivation, with production estimated at ~27 million tons (<http://www.ers.usda.gov/topics/crops>). Cotton is the leading cash crop in the United States, with annual business revenue stimulated by cotton exceeding 120 billion dollars to the economy www.cottoncounts.net. This accounts for approximately 35 percent of the total world's fiber used, half of which is exported

<http://www.ers.usda.gov/amber-waves/2013-june/crop>. In the U.S. alone the industry generates about 200,000 jobs and accounts for more than 25 billion in products and services (<http://www.ers.usda.gov/topics/crops>). China, India, the United States, and Pakistan accounting for more than 70 percent of global cotton production in 2013-14, while other important cotton producing countries are Australia, Brazil, the Africa Franc Zone, and Central Asia, and China, India, and Pakistan are expected to lead global cotton mill use and account for a combined 65 percent of world consumption in 2013-14 (Meyer et al., 2013).

Cotton leaf curl disease (CLCuD) complex is a debilitating disease of cotton that results in leaf curling, development of leaf-like enations on the undersides of leaves, overall stunting of the plant, and reduced yield and quality. The disease is caused by one or more whitefly-transmitted geminiviruses (genus, *Begomovirus*; family, *Geminiviridae*) that serve as the ‘helper virus’ (that replicates non-viral satellites, which are associated with virulence and symptom expression) for two different types of DNA satellite molecules of non-viral origin. The two types of satellites are referred to as betasatellites and alphasatellites, representing two different types of small, half unit sized (in comparison to the viral genome), circular ssDNAs. The satellites contribute to host defense suppression, leading to increased virulence of the helper virus resulting in systemic infection and the production of severe disease symptoms. In at least one instance, an alphasatellite has been shown to down-modulate helper virus-beta satellite symptoms and reduced betasatellite accumulation in tomato plants (Idris et al., 2011). In the Eastern Hemisphere, cotton leaf curl diseases of cotton occur in Africa, Pakistan, and northwestern (Punjab region) India. During the past three decades, Pakistan and India experienced two epidemics. The most recent epidemic resulted from the emergence of a resistance breaking strain that was able to overcome host-plant resistance in varieties developed to combat the first outbreak that occurred in about 1990 (Zafar and Brown, 2011; Zafar et al., 2003).

Worldwide, infection of cotton by whitefly-transmitted geminiviruses is most damaging when cotton plants become infected during early growth stages, compared to mid- or late- season stages. In Africa, Asia, and the Americas, early season infection of cotton by whitefly-transmitted viruses (genus, *Begomovirus*) has resulted in a total loss of the crop. There is great concern that CLCuD could spread from its current, extant geographical range in Pakistan and India, to other cotton growing areas of the world where, although the disease is not present, the whitefly vector is prevalent and the environmental conditions are suitable for disease establishment. Recently, this fear has proven well founded as CLCuD emerged in an ornamental species (malvaceous) and cotton in at least three locations in China, presumably transported from an unknown infected area (region of endemism).

Currently, no disease resistance is available to CLCuD in cultivated cotton or any other cultivated host species (vegetable or ornamentals). Even so, the reliance on genetic resistance for disease management has been the primary means considered for management in Pakistan and India. The ability of this highly differentiated virus complex and its satellites to break the resistant varieties developed and widely grown by 2001, during 2004 (Mahmood et al., 2003), and underscores the concern that the virus complex, which has a broad host range, may easily spread to new areas owing to incidental transport of asymptomatic plants harboring the virus. Efforts are underway in both India and Pakistan to identify genes in cotton germplasm sources that could yield viable resistance or at least tolerance to early season infection. In addition, some efforts are underway to pursue genetic engineering of cotton plants using virus-derived and non-virus derived resistance

with promiscuous satellites. These satellites can assist multiple viral species and strains to infect the host plant. Furthermore, both the ‘helper’ virus and the assisting satellites are known to undergo frequent recombination.

The host range of the virus-satellite complexes has been found to be astoundingly broad, spanning multiple plant families, even though it was thought early on to be restricted to cotton and wild malvaceous hosts.

Like other well-studied members of the geminiviruses, the viruses of the CLCuD complex are not seed transmitted. The CLCuD virus complex is transmitted from plant to plant through by the whitefly *Bemisia tabaci* (Genn.) [Hemiptera: s.o. Homoptera, Aleyrodidae) sibling species group in a circulative and persistent manner, and so once acquired, can be carried and transmitted for the life of the vector. Begomoviruses are transovarially (passed through the egg) or sexually transmitted, nor are they propagative (replicative) in the vector (albeit, one exception has been reported). And, mechanical transmission has not been demonstrated for any members of the CLCuD complex. The viruses can, however, be experimentally transmitted by grafting, and by inoculation of plants with infectious viral clones using biolistic inoculation, particle bombardment, or agroinoculation. Using the latter types of inoculation methods, it has not been possible to demonstrate the development of wild type disease symptoms in cotton, suggesting either that unidentified components remain unidentified, or that cotton is particularly recalcitrant to inoculation using other than the whitefly vector. *Nicotiana benthamiana* (Domin) is a useful bioassay host when infectious clones are available, owing to its general susceptibility to plant viruses; however, most haplotypes of the whitefly *B. tabaci* vector do not readily feed on this species, and so it is unreliable as a test plant when whitefly-mediated inoculation is desired.

Collectively, the severity of the disease, the ease with which the begomoviral-satellite complex(es) are transmitted by multiple biotypes and haplotypes of the whitefly vector, the ability of begomoviruses to undergo genetic changes in response to corresponding changes in the genetics of the host plant (documented, in particular for cotton), and the unusually broad host range of the viruses are causes for predicting that the expansion of the geographic range of members of the CLCuD complex. This expansion poses a considerable risk to a variety of high-cash value agronomic and horticultural crops outside the locales currently infected by these viruses.

The leaf curl disease of cotton crops is primarily managed by the use of pesticide treatments; often frequent, to kill the whitefly vector to reduce virus transmission by the vector. The lack of alternative control options has led to the profuse, and often overuse, of pesticides to reduce vector populations. This overuse of pesticides leads to the development of insecticide resistance in the whitefly vector, which subsequently undermines abatement of virus transmission to reduce damage and losses.

The development of molecular diagnostic methods that would facilitate the early detection of all members of this virus-satellite complex, including newly emerging ones, in plants and the whitefly vector has become essential.

It is feasible that the whitefly vector infesting a leaf curl non-host plant could harbor the virus complex, if ingested and acquired from an infected plant prior to dispersal to a non-host. In addition, symptomless plant hosts harboring the whitefly vector adults or immature instars that can develop to adulthood could pose a threat by introducing the virus and whitefly

simultaneously, as the infection goes initially unnoticed. Therefore, ornamentals or vegetable seedlings transported from high-risk areas could provide a route of unexpected route of entry of the leaf curl virus complex.

Finally, the ornamental industry has been known to distribute large numbers of plants to and from a wide range of geographical locales. Movement of asymptomatic, but infected ornamental plants and those infested with viruliferous whiteflies, particularly likely to be present in China, Europe, and the Arabian Peninsula, that engage in plant trade with regions that are not high risk regions for endemic CLCuD (e.g., Europe) and therefore poses no perceived threat, are potential pathways for the introduction of one or more species of the CLCuD complex. Other potential pathways could involve virus-infected or viruliferous, whitefly-infested ornamentals or vegetable seedlings transported through Canada from Europe to U.S. cotton-growing areas, or from Central America and the Caribbean region locales where cuttings or other propagated materials are grown from materials received from Africa, Asia, Europe, or the Middle East, and shipped to a variety of end-users in the U.S. and elsewhere.

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Cotton Leaf Curl Virus-Satellite Complex (Begomovirus, Geminiviridae): A whitefly-transmitted virus causing leaf curl disease of cotton, vegetable crops, and ornamentals

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I. Introduction

Cultivated cotton, *Gossypium* species (L.), has been a major source of food, feed, and fiber worldwide for at least 7,000 years. Globally about 32.6 million hectares are devoted to cotton cultivation, with recent production estimated at 25-27 million tons (Anonymous, 1997; Meyer et al., 2013; Sattar et al., 2013). Cotton is the leading cash crop in the United States, with annual business revenue stimulated by cotton exceeding 120 billion dollars to the U.S. economy alone www.cottoncounts.net. The crop accounts for approximately 35 percent of the total world's fiber used, and half of the U.S. crop is exported <http://www.ers.usda.gov/amber-waves/2013-june/crop>. In the U.S. alone the industry generates about 200,000 jobs and accounts for more than 25 billion in products and services (<http://www.ers.usda.gov/topics/crops>). China, India, the United States, and Pakistan accounting for more than 70 percent of global cotton production in 2013-14 (Meyer et al., 2013), and China, India, and Pakistan are expected to lead global cotton mill use and account for a combined 65 percent of world consumption in 2013-14, while other important cotton producing countries are Australia, Brazil, the Africa Franc Zone, and Central Asia (Meyer et al., 2013).

Both abiotic (drought, flooding, heat or cold stress) and biotic stresses are responsible for yield losses in cotton production, with insects, plant fungal and viral pathogens, and weeds contributing to reduced production. This report will address viral pathogens given the rising importance of whitefly-transmitted viruses in cotton and their impending spread from a major center of origin in the Indian Subcontinent elsewhere where cotton is produced.

A number of plant virus-like symptoms associated with decreased yield and quality, even loss of the entire crop have been described in cotton from different parts of the world. Over 20 virus-like diseases of cotton have been described (Brown, 1990, 1992; Kirkpatrick and Rothrock, 2001), but only a few have been confirmed to be of viral etiology, including the whitefly-transmitted geminiviruses, *Cotton leaf crumple virus* (CLCrV) (Brown and Nelson, 1984, 1987; Idris and Brown, 2004), several members of the Cotton leaf curl complex (India and Pakistan) (Briddon et al., 2000; 2001; Mansoor et al., 2003). Of the most economically important cotton diseases for which an etiological agent has been established, members of the genus, *Begomovirus* (family, *Geminiviridae*) (Briddon and Markham, 2000; Brown, Idris and Brown, 2004) and a luteovirus belonging to the genus *Polerovirus* (Corrêa et al., 2005; Distéfano et al., 2010; Silva et al., 2008) have proven most important. Among these, the begomoviruses are the most destructive and pose the primary plant viral threat where cotton is grown (Briddon and Markham, 2000; Brown, 1992; 2002; Mansoor et al., 2006; Idris and Brown, 2002; 2004).

Geminiviruses are circular, single-stranded (ss) DNA viruses with small genomes encapsidated in twinned, icosahedral (geminata) particles. They are transmitted by an homopteran (Order, Hemiptera) vector. Geminiviruses infect either monocotyledonous or dicotyledonous plants, and are taxonomically separated into seven genera based on insect vector, genome organization, and host range (2012.018a-pP.A.v4.Geminiviridae.pdf available at ICTVonline.org/virusTaxonomy). Among the family *Geminiviridae*, the genus *Begomovirus* contains the greatest number of taxa,

and is economically most important because they have emerged in cultivated crop species as agriculture has expanded in the last two centuries. They are widespread in wild or unmanaged, endemic or introduced, eudicot hosts. Begomoviruses infect crop species throughout the tropics, subtropics, and temperate regions with mild climates such as the Sunbelt States of the United States. Begomoviruses are transmitted between plant hosts by the whitefly *Bemisia tabaci* (Genn.) sibling species group [Aleyrodidae; Hemiptera] (Brown, 2010).

Taxonomically, begomoviruses are grouped into two major, divergent taxonomic clades: those originating from the Old World (OW) or those originating from the New World (NW). The NW begomoviruses have a genome consisting of two components of approximately 2,600 nucleotides (nt) in size, referred to as the DNA-A and DNA-B component, respectively. Each component is encapsidated in a separate icosahedral particle such that each virus particle contains either a DNA-A or DNA-B component, and both are required for systemic infection of the plant host. This type of virus occurs in both the NW and OW. Monopartite begomoviruses have a single genomic component, referred to as DNA-A. Monopartite viruses, thus far, occur only in the OW, and so it has been hypothesized that geminiviruses have their origin in the Eastern Hemisphere (Briddon et al., 2010).

Satellites associated with ‘helper’ begomoviruses

Satellites are defined as viruses or nucleic acids (DNA or RNA) that depend on a helper virus for their replication but lack extensive nucleotide sequence identity to their helper virus and are dispensable for its proliferation (Murant and Mayo, 1982). The majority of plant viral-associated satellites known previously consist of RNA, are associated with viruses with RNA genomes, and have variably discernable effect on the symptoms caused by the helper virus in plants. In contrast, most but not all monopartite begomoviruses associate with a small, non-viral (~1350 nt) circular ssDNA molecules, referred to as betasatellites. Helper viruses vary in the degree to which they require betasatellites to systemically infect the host and cause wild type disease symptoms. Betasatellites are approximately half the size of their helper virus genome and have a highly conserved structure, despite their sequences sharing as little as 45% nt sequence identity (Briddon et al., 2003; 2008). They encode a single gene, beta C1 (β C1), in the complimentary sense, are rich in adenine, and contain a 80-100 nt fragment that is highly conserved among all betasatellites. The conserved region is referred to as the satellite conserved region (SCR) (Briddon et al., 2003; Briddon et al., 2001). The role of betasatellites in the infection cycle has been attributed to the product of the single gene they encode (β C1). It has been shown to be a pathogenicity (symptom) determinant (Saeed et al., 2005; Saunders et al., 2004), and functions as a suppressor of post-transcriptional gene silencing, which facilitates systemic infection of the host (Cui et al., 2005; Saeed et al., 2007).

A second type of satellite, the alphasatellites, was first identified as a class of molecules termed DNA-1, but they are now referred to as alphasatellites (Mansoor et al., 1999; Saunders et al., 2000). The alphasatellites comprise a group of closely related ssDNA molecules that encode a single protein, a rolling-circle replication initiator protein, the replication-associated protein (Rep) that autonomous replication. Their Rep protein shares an evolutionary relatedness to nanovirus Rep. For all other functions, alphasatellites depend on the helper begomovirus, including movement within the plant, and whitefly-mediated transmission (Mansoor et al., 1993). Alphasatellites share no significant levels of sequence identity to their helper begomoviruses except for a predicted

hairpin structure within the loop, referred to as the nonanucleotide sequence, the cleavage site for rolling circle replication (Saunders et al., 2000; Saunders and Stanley, 1999). They rely on the helper virus for encapsidation, vector transmission, and perhaps indirectly for other critical functions in the infection cycle of the virus. The precise role(s) in the disease complexes that infect cotton has not yet been clarified (reviewed in Sattar et al., 2013). They are not thought to be essential for systemic infection of the helper virus.

Because disease symptoms can be reproduced in certain host plants inoculated with different combinations of helper viruses and betasatellites (Saunders et al., 2004), the assumption has been that alphasatellites are of little etiological consequence. However, recent evidence suggests that the alphasatellites can contribute an important role in disease severity by modulating the virulence of the helper virus, based on reduced symptom severity and decreased levels of betasatellite DNA when the alphasatellite was co-inoculated with the helper- betasatellite complex (Idris et al., 2011). In addition an alphasatellite Rep gene has been shown to suppress host-plant induced gene silencing in the youngest leaves, a phenomenon mediated by the alphasatellite encoded Rep and helper viral Rep and C4 proteins (Nawaz-ul-Rehman et al., 2010). The three most widespread begomoviral pathogens are those that comprise the Cotton leaf curl disease complex (CLCuD) (Briddon et al., 2001; Mansoor et al., 1993; 1999; 2003a,b) in India and Pakistan (and recently introduced into China), the *Cotton leaf curl Gezira virus* (CLCuGV) (Idris and Brown, 2002; Idris et al., 2005) which is present throughout sub-Saharan Africa and Arabia, and *Cotton leaf crumple virus* (CLCrV) (Brown and Nelson 1984; Idris and Brown, 2004; Dickson et al., 1954), which is endemic to North and Central America.

Among the major groups of begomoviruses known to infect cotton, the Asian cotton leaf curl disease complex from Pakistan and India (Indian subcontinent) is considered to be the greatest current threat, owing to the wide diversity of species and strains that comprise the complex (citation?), the proven ability to overcome resistance in cotton varieties developed to manage the disease (citation?), , and evidence that it has already spread on the Asian continent from its origin in the Punjab (citation?). The second most important begomovirus is CLCGeV, which is apparently widespread in the African cotton belt. The virus was also shown to be capable of re-distribution from its region of origin in Africa to Pakistan (citation?). There are no known resistant varieties to any cotton-infecting begomoviruses adding to the risk they pose to worldwide cotton production.

Indian Subcontinent - Asia

In Asia, CLCuD is caused by a complex of diverse begomoviruses that are endemic to the Indian subcontinent. To date, numerous begomoviruses and associated betasatellites have been cloned and characterized (to varying degrees) from symptomatic cotton plants. These include *Cotton leaf curl Alabad virus* (CLCuAV), *Cotton leaf curl Burewala virus* (CLCuBuV), *Cotton leaf curl Kokhran virus* (CLCuKoV), *Cotton leaf curl Multan virus* (CLCuMuV), *Cotton leaf curl Rajasthan virus* (CLCuRaV) and *Cotton leaf curl Shahdadpur virus* (CLCuShV). A recombinant derived from CLCuKoV and CLCuMuV have been cloned from cotton and characterized (Sattar et al., 2013). In addition, *Papaya leaf curl virus* (PaLCuV) and *Tomato leaf curl Bangalore virus* (ToLCBaV) and their associated betasatellites have been identified in cotton in the Punjab region (citation?). In India, *Cotton leaf curl Bangalore virus* (CLCuBaV) was identified in southern India where it is associated with kenaf curl betasatellite (KeLCuB). This satellite also was detected in kenaf (*Hibiscus*

canabinus L.) plants in India (Paul et al., 2008; Roy et al., 2009; Sattar et al., 2013).

Cotton leaf curl disease in Pakistan was first reported during 1967 near Multan (Hussain and Ali, 1975). This disease is characterized by an upward curling of leaves, thickening of veins and laminar outgrowth on underside of the leaves referred to as enations (Mahmood, 1999; Khalid et al., 1999; Akhtar et al., 2002a). Attention was drawn to the disease in 1973 when leaf curl symptoms became prominent in several important cotton varieties, including 149-F and B-557. Symptoms were observed late in the season and only on the flush growth. By 1987, the incidence increased to as high as 80% in some fields, damaging 60 hectares of the crop in the Multan District. During 1991, leaf curl disease affected 14,000 hectares in Multan, Khanewal, and Vehari Districts, and by 1992, 48,500 hectares were infected. During the 1993 season the disease spread to the entire cotton belt of the Punjab damaging 889,000 hectares. The increased incidence in leaf curl disease during the mid 1970's and early 1980's has been attributed to the decline in popularity of the smooth leaf cotton varieties, because they became highly susceptible to jassid infestation. This caused production varieties to shift to hairy or hirsute leaf types, which were not susceptible to jassid (R. Chaudhry, ICAC; personal communication). The hirsute varieties were then found to stimulate cotton infestation by the cotton whitefly *B. tabaci*, which preferred the hirsute varieties over the smooth leaf varieties previously grown. The subsequent use of insecticides to control the whitefly resulted in the development of insecticide resistance, and whitefly populations spiraled out of control. The unprecedented whitefly infestations led to the rapid spread of the leaf curl disease agent (then of unknown etiology), from near Multan, and then to other cotton growing areas of Pakistan (Hussain and Ali, 1975), and finally into India. The virus found to be responsible for this outbreak was identified as the previously undescribed whitefly-transmitted geminivirus (genus, *Begomovirus*), *Cotton leaf curl Multan virus* (CLCuMV) (Briddon et al., 2001; Mansoor et al., 1993, 1999).

The effects of the disease on production, during 1991-1999, proved to be disastrous. Pakistan reported that in the first year, the epidemic reached full scale, yields declined by one million bales from a record production of 12.82 million bales. By 1994-1995, the epidemic reduced yields to 7.9 million bales (Anonymous, 1997). After 1995, leaf curl disease of cotton occurred annually and the industry in Pakistan was debilitated. Breeding efforts were undertaken to develop resistance varieties to combat the disease, and production returned to pre-epidemic levels. However, during 2001-02, a second outbreak occurred. This second outbreak began in the Burewala territory in the Punjab Province where the disease affected the cotton varieties that had been developed to combat infection by CLCuMV, the predominant causal agent of the 1994-95 epidemic (Mahmood et al., 2003; Mansoor et al., 2003a,b). Sequencing of viral isolates associated with the Burewala outbreak revealed the predominance of an emergent new species, referred to as *Cotton leaf curl Burewala virus* (CLCuBV).

The viral genome contains sequences present in two species, one associated with the 1990's pandemic, CLCuMuV and a second species that had been detected but was not widespread, CLCuKoV (Amrao et al., 2010a). The new recombinant, CLCuBuV was shown to have mutated C2 protein, in that the virus lacks a complete C2 open reading frame (ORF), which in other begomoviruses encodes a protein involved in suppression of the plant host gene silencing apparatus that suppresses begomoviral infection of the plant (Amrao et al., 2010b). This suggests that the resistance-breaking phenomenon is directly or indirectly associated with this gene product. Following its spread throughout all of Pakistan, CLCuBuV spread to the Indian Punjab, where it

infected varieties resistant to the Multan complex, resulting in extreme crop losses in both countries in 2009-2010. It has become the predominant begomovirus in northern India (Rajagopalan et al., 2012).

This recombinant virus originating in Burewala proved to be more virulent than CLCuMV when infection occurred at the early growth stages (Arshad et al., 2006). CLCuBV subsequently spread rapidly to the most productive area in the central Punjab (Khanewal, Multan, Lodhran, Vehari, Bahawalnagar, Bahawalpur) where it reduced yields significantly during 2007-2008. Studies have shown that all the varieties that were resistant to CLCuV-Multan were found to be susceptible to infection by the Burewala strain of the virus (Mahmood et al., 2003; Tahir et al., 2004). Presently, this recombinant Burewala virus has spread to all cotton-growing regions of Pakistan, and into the Indian Punjab, a major cotton production area for India.

Due to the geographical proximity of the Punjab regions of Pakistan and India, and the direction of the prevailing winds, it is hypothesized that the Multan and Burewala CLCuD epidemics first caused by CLCuMV and then by CLCuBV, likely spread eastward from Pakistan into northwestern India from where it then moved further into the other northwestern state (based on the distribution of the disease). Further evidence taken from the more recently discovered species, CLCuMuV, CLCuKoV and CLCuBuV first identified in Pakistan, also are now present in cotton in northwestern India (Rajagopalan et al., 2012; Zaffalon et al., 2011), whereas, several begomoviruses identified in cotton in India have not been detected in Pakistani cotton. For example, CLCuRaV occurs widely in cotton in India but has only been detected in exotic cotton species maintained in the 'living herbarium' in Multan (Nawaz-ul-Rehman et al., 2010). However, the latter virus has been detected infecting tomato in Pakistan (Shahid et al., 2007), and so could pose a threat to the Pakistan cotton crop at some point in the future. A differing few holds that virus-infected or whiteflies harboring the virus but associated with seedlings that have been moved between the locations were the initial source of the virus.

Some have speculated that CLCuRaV spread from India into Pakistan, but it seems more likely that it has not been selected for as an important begomoviral species infecting cotton in Pakistan because of the specific genetic context of cotton varieties grown in there, which instead facilitated the ready establishment of two other apparently more fit species that became predominant simultaneously in response to the genetic background used in breeding programs. Similarly, ToLCBaV has been detected in cotton in India, but it is not prevalent in this host. This could suggest that it infects cotton only rarely, i.e. is not an effective begomoviral pathogen of cotton. Analogously, the bipartite *Tomato leaf curl New Delhi virus* (ToLCNDV) has been reported in cotton from India (EF063145) but it is not commonly found (citation?). Even so that the ToLCNDV betasatellite is known to have contributed a fragment (SCR region) to create the recombinant Burewala helper virus-associated betasatellite, ToLCNDV and/or its satellite (promiscuously associated with another cotton-infecting helper virus) have clearly intermingled with the monopartite virus complexes found in cotton in Pakistan prior to the outbreak caused by the CLCuBV resistance-breaking strain.

Studies of begomovirus diversity in cotton in India since the emergence of CLCuBV indicate that CLCuBuV and CLCuRaV are the predominant species in cotton there (Rajagopalan et al., 2012). This differs from the current situation in Pakistan where only CLCuBuV is widespread (Amrao et al., 2010b). Also, in India, CLCuMV has been detected in malvaceous species other than cotton, including species of *Hibiscus* grown as ornamentals, and fiber crops such as *Hibiscus*

cannabinus and *Hibiscus sabdariffa* (Das et al., 2008; Paul et al., 2008; Roy et al., 2009). These reports confirm that this virus is not restricted to cotton and can be harbored by wild hosts and other cultivated species.

In both India and Pakistan, begomoviral species that infect cotton have only recently been detected in other wild and cultivated malvaceous hosts, including non-malvaceous vegetable and ornamental hosts. Earlier studies demonstrated that okra was a host of the Multan virus (Zhou et al., 1998), implicating non-cotton hosts of the leaf curl virus. Recently, Ur-Rehman et al. (2013) reported CLCuBV infecting luffa plants in Pakistan, raising the likelihood that cotton-infecting viruses in the subcontinent could infect species and even plant families far beyond the currently recognized host range. Because of CLCuBV's wide host range, and the ease with which it is transmitted by its widespread whitefly vector, these viruses could spread from the Indian subcontinent to establish in other cotton and vegetable producing areas, including Africa, Australia, Latin America, and the United States.

Africa

Leaf curl in Africa was first reported in Nigeria in 1912 (Farquharson, 1912), followed by Sudan (Golding, 1930) and Tanzania (Kirkpatrick, 1931). When leaf curl symptoms were observed in Pakistan during the 1970's (Hussain and Ali, 1975) the name 'cotton leaf curl' was erroneously adopted, because it was not yet recognized that the viruses in Africa were quite different from those species endemic to Pakistan and India.

One virus species predominates in African cotton. *Cotton leaf curl virus-Gezira virus* (CLCuGV) (Idris and Brown, 2002) and its betasatellite appear to be widespread throughout the cotton belt in sub-Saharan Africa. The first isolate of this virus to be characterized at the molecular level was the Gezira isolate from Sudan. As with the Asian leaf curl viruses, CLCuGV is associated with a betasatellite, *Cotton leaf curl Gezira betasatellite* (CLCuGB). The virus and associated satellites are genetically and phylogenetically distinct from those occurring on the Indian subcontinent (Idris and Brown, 2002; Idris et al., 2005). Recent studies have demonstrated that CLCuGV is distributed from central Africa to Jordan where it infects cotton, okra, hollyhock and *Sida* spp. (Tahir et al., 2011), and in various cultivated malvaceous hosts throughout the Arabian Peninsula (Idris et al., 2013).

Although several begomoviruses have been described in other malvaceous hosts in sub-Saharan Africa, only CLCuGV has been detected infecting cotton in the region (Idris and Brown, 2002; Tahir et al., 2011). Other begomoviruses causing leaf curl and other diseases in okra and tomato are widespread in West Africa and could possibly infect cotton and ornamental species. Thus far, only a few begomoviruses, other than those that are associated with cassava, have been characterized from sub-Saharan Africa. These latter viruses endemic to the region may therefore become of potential importance to cotton production in Africa (Sattar et al., 2013).

Americas

The third begomoviral species infecting cotton is *Cotton leaf crumple virus* (CLCrV), a bipartite virus of New World origin. It is represented as a group of closely related variants. Symptoms of the disease are foliar discoloration, leaf crumpling, shortening of internodes, and stunting in both cotton, and in experimentally and naturally infected common bean [*Phaseolus vulgaris* (L.)]. Disease severity is dependent upon plant age at time of infection. Damaging outbreaks of cotton leaf crumple disease may be exacerbated by rationing, a practice in which cotton is pruned and

allowed to re-grow the following year. In most years, infection occurs after cotton plants have developed to the 10-14-leaf stage, so CLCrV has not been considered an economically important disease of cotton. In years when whitefly populations build to high levels early in the season, plants become infected during the early growth stages and damage can be extensive. However, management is usually confined to controlling the whitefly vector to reduce virus transmission and secondary spread. Several virus-tolerant lines have been selected from efforts to develop disease resistant cotton varieties using 'Cedix variety' as the source of introgressed genes (Idris and Brown, 2004).

Genetic diversity among cotton-infecting begomoviruses

In the Old World (OW), two major groups or clades of cotton-infecting begomoviruses are recognized, those from Asia and a divergent group from Africa. These two major clades are highly divergent from the CLCuV species endemic to the Americas, suggesting that all three groups of viruses independently adapted and diversified with cotton.

In Asia, the CLCuD complex comprises a large number of begomovirus species and strains (at least seven), together with a number of associated DNA beta and alpha satellites. The first study of the genetic diversity of begomoviruses associated with CLCuD found four begomovirus variants infecting cotton in Pakistan (Zhou et al., 1998), with the predominant virus being *Cotton leaf curl Multan virus* (CLCuMV). The other three viruses are classified as the distinct species: CLCuMV, *Cotton leaf curl Alalabad virus* (CLCuAV) and *Cotton leaf curl Khokhran virus* (CLCuKV). *Cotton leaf curl Shahdampur virus* (CLCuShV) has been identified in Pakistan only recently (citations?). Both CLCuBuV and CLCuShV have recombinant genomes, with sequences derived from CLCuKoV and CLCuMuV. In northwestern India, many viruses identified in Pakistan occur together with *Cotton leaf curl Rajasthan virus* (CLCuRaV) (Kirthi et al., 2004). This species has now been identified in Pakistan from cotton and tomato (Nawaz-ul-Rehman et al., 2010; Shahid et al., 2007). Studies have identified two additional begomoviral species in cotton: *Papaya leaf curl virus* (Mansoor et al., 2003b) and *Tomato leaf curl Bangalore virus* (Kirthi et al., 2004). In summary, at least nine begomoviral species are

capable of infecting cotton in either Pakistan and/or India (Sattar et al., 2013), with *Cotton leaf curl Burewala virus* (CLCuBV) and its associated satellite, the latest species to be recognized and to become widespread by 2004.

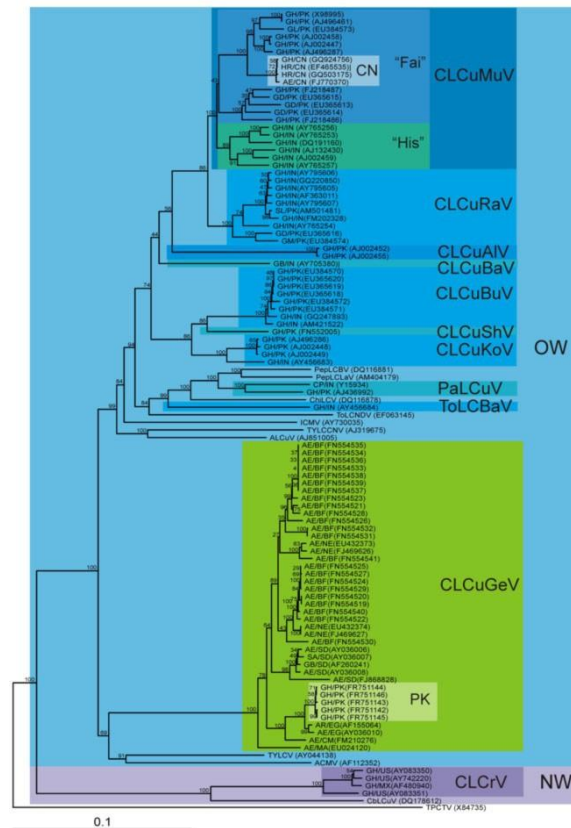


Figure 1. Phylogenetic tree showing the relationships between cotton-infecting begomoviruses in the Old and New World, illustrating the diversity of viruses originating in the Eastern Hemisphere, compared to the narrow genomic diversity of cotton viruses endemic to the Asian Subcontinent, based on a phylogenetic comparison of viral genomic sequences (from Sattar et al., 2013).

The African cotton-infecting begomovirus isolates known thus far are only distantly related to those of Asian origin, as is illustrated by the complete genome sequences (Figure 1; taken from Sattar et al., 2013). In Sudan, where the leaf curl disease affected cotton production during the early part of the 20th century, one predominant begomovirus species CLCuGV has been associated with the disease there (Idris and Brown, 2002). In studies using infectious clones constructed from viral DNA isolated from *S. alba*, the inoculated cotton plants did not become infected even though *S. alba* plants were systemically infected, suggesting that *S. alba* is either a less recalcitrant host to experimental inoculation than cotton, or that slight differences in genome sequences account for host range differences. Recently, CLCuGV was identified for the first time in Burkina Faso, and several other begomoviruses were detected in the West African cotton belt infecting malvaceous hosts, including cotton, okra and *Sida alba* (Tiendrébéogo et al., 2010). In addition, CLCuGV occurs throughout the Arabian Peninsula in various malvaceous hosts. The genome sequences of the isolates from Arabia share 88-93% nt identity to previously reported strains of CLCuGV. In addition, the betasatellite shared 60% nt identity with *Cotton leaf curl Gezira betasatellite* reported from the Nile Basin and sub-Saharan Africa. Nt sequence comparisons revealed that one alphasatellite shared 88% nt identity with Cotton leaf curl Gezira alphasatellite (DNA-1 type), while the second satellite shared the highest nt identity (64%) with Ageratum yellow vein Singapore alphasatellite (DNA-2 type) from Oman and Singapore. The combined high variability and relatively low nt identities of these molecules with previously reported satellites suggest they are endemic, and that they are not the result of recent introductions (Idris et al., 2013). The CLCuGV also has been found in southern Pakistan, and the genome sequence for the Pakistani isolate is so similar to those from Sudan that it appears to have been recently introduced there (Tahir et al., 2011). Finally, in Egypt, a distinct begomovirus has been identified in the malvaceous host, hollyhock, and has been named *Hollyhock leaf crumple virus* (Idris et al., 2002). That this virus infects hollyhock and other malvaceous species suggest that it may be able to cause disease in cotton but to date it has not been identified in cotton and whitefly-transmitted virus-like symptoms in Egypt have been relatively rare (J.K. Brown, personal observation).

In the Americas, a single species consisting of several strains or variants (mostly owing to recombination) prevails, spanning the southwestern U.S. (AZ, CA and TX), northwestern and southwestern Mexico, and Guatemala. In Puerto Rico and the Dominican Republic, virus-like symptoms have been observed and confirmed to be begomoviruses, but they represent different species whose closest relatives are found in endemic, uncultivated malvaceous species. Although a number of variants occur in the United States (AZ, CA and TX), Mexico, and Guatemala they comprise a single species (0-4% nt divergence across the genome) (Brown and Nelson, 1984; Idris et al., 2004). A comparison of the CLCrV ORFs with those of closely related begomoviruses indicated that the CLCrV AC3 ORF shares a maximum percentage nt identity with *Potato yellow leaf mosaic virus* (PYMV), at 87%. Although the CLCrV AV1 shares 83 % nt identity with its close relative, *Sida yellow vein virus* (SiYVV), comparisons at the amino acid (AA) level indicate that the AV1 sequence for the two viruses are 93% identical. Similarly, CLCrV AC2 and AC3 ORFs shares high nt identity with SiYVV, at 82-83%, and a correspondingly high amino acid sequence identity (Idris and Brown, 2004).

II. Signs and Symptoms

Cotton leaf curl symptoms were first reported in Nigeria in *G. barbadense* cotton (Farquharson, 1912). In 1924, similar symptoms were reported to be widespread in the Sudan cotton crop

(Golding, 1930), and subsequently, in 1926 the disease broke out in Tanzania (Kirkpatrick, 1931). When leaf curl symptoms were observed in Pakistan cotton crops during 1967, the ‘leaf curl disease’ name was incorrectly adopted based on the ‘leaf curl’ symptom phenotype, irrespective of the particular agent(s), later shown to be highly divergent species. Leaf curl symptoms in Sudan and Pakistan are characterized as curling of the leaf margins, either upward or downward, and a crinkled appearance of the leaves, now referred to as ‘enations’, consisting of leafy tissue developing directly from the leaf veins. The veins of the affected leaves become thickened and more pronounced on the underside (Figure 2a-c). In Africa, two types of vein thickening are reported for CLCuGV, small vein thickening and main vein thickening. Small-vein thickening is the most common phenotype, and is characterized by small green bead-like thickening on the young leaves (Figure 3a-d). The irregular thickening gradually extends and coalesces to form a continuous reticulation of the small veins. Main vein thickening first appears near the leaf margin, and extends inward to form a network of dark green thickened main vein. In extreme cases, leaves form cup-shaped, and leaf-like outgrowths appear on the underside of the leaves. Tarr (1951) reported spirally twisted petioles, fruiting branches and tall stems and elongated internodes in *G. barbadense*. Most varieties in Africa infected by CLCuGV exhibit dwarfing, overall stunting, and reduced boll number and boll weight.



Figure 2a-c. (a) Leaf curl disease symptoms in cotton, (b) compound foliar enation on the underside of a leaf, and (c) cup-shaped veinal-enation on the leaf underside, all from Pakistan.





Figure 3a-d. Symptoms of CLCuGV from Sudan in (a) cotton plants, (b) underside of cotton leaf, (c) hollyhock plant, lower leaf surface, and (d) hollyhock plant, upper surface.

Symptoms caused by CLCrV in the Americas are fairly similar from one location to another but differences are noted with certain varieties. Cotton (Figure 4a,b) and kenaf (Figure 4d) plants infected in the seedling stage develop severe leaf curling, blistering, and crumpling on the newest growth. Symptoms persist in the leaves throughout the season (Figure 4a). Some varieties also develop yellow-green mosaic symptoms (Figure 4b). In plants infected at later growth stages, leaf symptoms are mild or absent, unless flush growth is stimulated, and then all of the latter leaves will develop typical leaf crumple symptoms. Flower petals (Figure 5c) and bolls also develop symptoms, particularly in plants inoculated prior to the 8-10 leaf stage. Yield and fiber quality are reduced substantially (Brown et al., 1987).



Figure 4a-d. Symptoms of *Cotton leaf crumple virus* infection in (a) cotton plants in Arizona, USA (b) cotton plants in Caborca, Mexico, (c) flower petals, and (d) in kenaf plants, Texas, USA.

III. Spread and Risk

Members of the complex are not seed transmitted, a property consistent with other well-studied geminiviruses. The leaf curl virus complex is spread by the whitefly *B. tabaci* sibling species group in a circulative, persistent manner. Begomoviruses are not transovarially or sexually transmitted (with one possible exception, *Tomato yellow leaf curl virus*, from Israel), nor has mechanical transmission been demonstrated for the leaf curl complex viruses.

The *B. tabaci* sibling species comprises a cryptic (morphologically indistinguishable) group of *Bemisia* sibling species that may exhibit restricted gene flow (or not), indicating that speciation has occurred or is impending within this whitefly. Members of sibling species group exhibits different biological characteristics, harbors distinct suites of endosymbionts, have a variety of phenotypes, and are adapted to different environments. The group as a whole colonizes over 500 species of plants, however, the host range of a single haplotype is not thought to be so extensive owing to their propensity to adapt to and prefer certain suites of hosts. Those populations that have been characterized to at least some extent biologically, are referred to as ‘biotypes’ whereas, those for which only a molecular marker (mtCOI) sequence is available, are referred to as “haplotypes”.

Virus-satellite complexes can be experimentally transmitted by grafting, and by inoculation of plants with infectious viral clones using biolistic inoculation, particle bombardment, or agroinoculation. The latter types of inoculation methods do not necessarily result in the development of wild type disease symptoms in cotton. *Nicotiana benthamiana* (Domin) is a useful bioassay host when infectious clones are available, owing to its general susceptibility to plant viruses.

Pathways for entry into the United States include cuttings and propagative host materials. These methods are regulated by USDA-APHIS under the authority of the Plant Protection Act with regulations in 7 CFR Part 319, which prohibit or restrict entry of certain plants and plant products to prevent introduction of plant pests and pathogens into the United States. All cotton (*Gossypium* sp.), okra (*Abelmoschus esculentus*), and tomato (*Solanum lycopersicum*) plants are prohibited, except for the seed. Hibiscus (*Hibiscus* sp., and *Hibiscus cannabinus*) is prohibited from Africa, Brazil and India, and also must meet Federal Order effective May 11, 2011 (specifically for the Importation of host material of *Anoplophora chinensis* (Forster), the Citrus Longhorned Beetle and *Anoplophora glabripennis*, Asian Longhorned Beetle). These materials are subject to size restrictions and must undergo 2-year post-entry quarantine. *Cucurbita* species are generally allowed, although cuttings have not entered the United States from the Middle East or China during 2011-2013 (citation?).

Natural spread of the leaf curl disease is therefore mainly by the whitefly vector (*B. tabaci*). It can complete the transmission cycle from the acquisition of the virus to infection of a new host plant, within 6.5 hours (citation). *B. tabaci* is capable of establishing high population levels, particularly in crops grown under irrigated, arid conditions in both field and greenhouse environments. In addition, this whitefly has the potential to colonize a wide range of dicotyledonous species, including vegetable and fiber species of great importance to worldwide agricultural production. Studies have shown that there are numerous variants of *B. tabaci*, referred to as biological types (biotypes) that can differ with respect to fecundity, feeding damage, insecticide resistance, and virus transmission efficiency (competency) (Bedford et al., 1994; Brown and Bird, 1992; Brown et al., 1995; Brown, 2010, and references therein; see refs in Brown, 2010; Maruthi et al., 2002).

Recent introductions:

Based on knowledge of the diversity of viral genomes and their beta-alphasatellite complexes that are associated with cotton crops in different locations, it is possible to ascertain whether a virus is endemic or recently introduced from its zone of endemism. The introduction of exotic, genetically divergent begomovirus-satellite complexes to cotton-growing areas where the viruses are not endemic has great potential to cause outbreaks because tolerance or resistance to the exotic viruses is not likely to exist in local germplasm. This is because resistance has been selected in the presence of the endemic virus and whitefly vector populations. The demonstrated high likelihood for recombination between helper virus genomes and beta and alpha satellites, as well as reassortment of satellite-helper complexes, makes it possible for new variants to arise. Presently, there is evidence of the spread of cotton-infecting viruses from their endemic habitat to new locations.

For example, in southern Pakistan, CLCuGV was detected infecting cotton plants for the first time (Tahir et al., 2011). The percentage nucleotide (nt) sequence identities between the Sudan and Burkina Faso isolates and the Pakistan isolates are greater than 95%, suggesting that the Pakistan isolate is a recent introduction from Africa. Thus far, it has been found south of the Punjab region, suggesting it has been transported there from Sub-Saharan Africa by human activity (Karachi is a major port, for example) (Figure 5, taken from Tahir et al., 2011). CLCuMV, which caused the epidemic in the Punjab regions of Pakistan during the 1990's, has been reported in two Chinese provinces, Guangdong and Guangxi. The first report occurred in Guangzhou

(Ghangdong Province) on *Hibiscus rosa-sinensis* plants (rose mallow) (Mao et al., 2008). In 2009, CLCuMV and its beta satellite were identified in cotton in Guangxi (Cai et al., 2010). During 2010, two additional isolates that resemble the

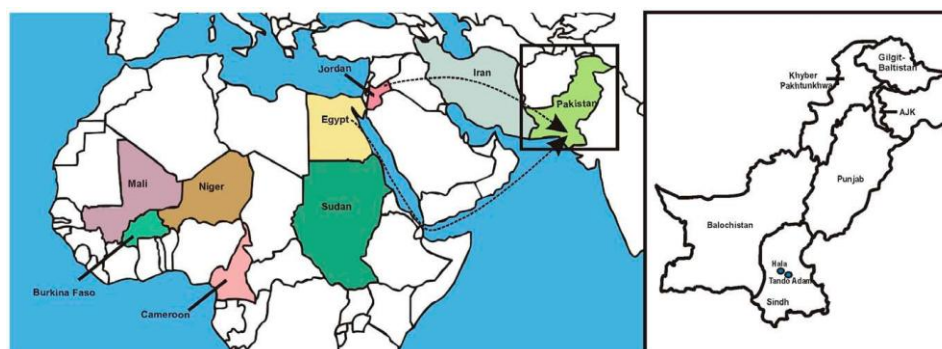


Figure 5. Proximity of cotton growing regions in Africa and Indian-subcontinent, illustrating the ease with which plant viruses can be moved by human activity involving exportation/importation of plants between the continents (taken from Tahir et al., 2011).

Multan species were sequenced from okra (*Hibiscus esculentus*) and cotton plants (GenBank Accession no. GU574208, Xie et al., 2010; <http://www.ncbi.nlm.nih.gov/pubmed/23115967>, Lai et al., 2012) and *Malvastrum arboreus* (Turks cap) (Tang et al., 2013) exhibiting leaf curl symptoms, both from Guangzhou. The former isolates consisted of CLCuMV and CLCuM DNA beta (Tang et al., 2013). The latter CLCuMV isolates were reported to have a defective betasatellite, a feature also reported for CLCuGV from Arabia (Idris et al., 2013). The significance of these defective betasatellites is not yet known, and additional information is required to understand the etiology and possibly additional complexity of the leaf curl isolates now circulating in China. However, it is thought that the CLCuMV-betasatellite complex was transported from Pakistan to the Gulf States on *H. rosa-sinensis* plants from where it was later imported into China on infected cuttings or plants. These observations suggest that the exportation of the latter virus likely occurred before the

emergence of the Burewala virus in Pakistan, and therefore has been in China for a longer period of time than had been documented.



Figure 6a,b. Photos showing *Hibiscus rosa-sinensis* (L.) plants (rose mallow) (left), and *Malvaiscus* (also, *Malvaviscus*) *arboreus* var. *drummondii* (Torr. & Gray) Schery (Turks cap) (right) plants that are hosts of the *Cotton leaf curl Multan virus* complex originating in Pakistan, and recently introduced to China.

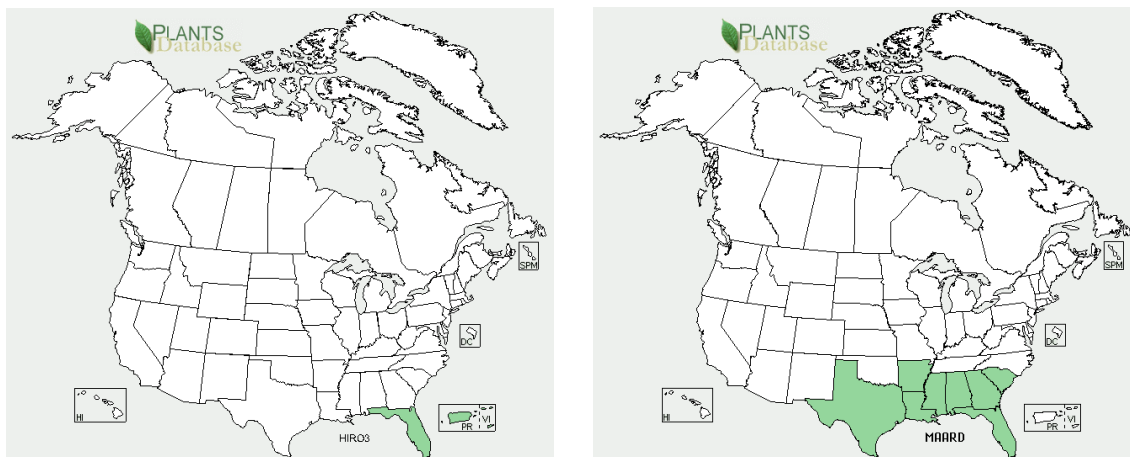


Figure 7. Maps illustrating the distribution of hibiscus (*H. rosa-sinensis*) (left) and Turks Cap (*M. arboreus*) (right) in the southern United States and/or Puerto Rico, however, *H. rosa-sinensis* is not restricted to Florida, as is shown in this map, but is grown in many locations in the U.S. owing to its wide distribution through the nursery trade. These ornamental species are two of many ornamental species that are known or suspected hosts of CLCuD complex (taken from <http://plants.usda.gov/>; http://www.wildflower.org/plants/result.php?id_plant=MAARD).

IV. Detection and Identification

Knowledge of when and where mutations occur and/or are fixed in the viral- satellite populations by host (or whitefly vector) selection will alert cotton producers and breeders to the potential emergence of a potentially damaging new threat to currently cultivated varieties and cotton germplasm under consideration in genetic improvement programs. The ability to detect subtle changes in the viral genome and population structure in near real time will warn of the impending spread of the leaf curl-satellite complexes from their regions of endemism to exotic ones where extensive damage would likely occur in unprotected cotton cultivars (those not bred for resistance or tolerance to exotic viral pathogens). Employing this multifaceted, proactive approach to viral population structure analysis will provide the most proactive means of initiating proper actions and subsequently, recovery from the effects of disastrous outbreaks, and identify potential resistant germplasm.

Currently, the virus and satellite complexes may be detected by polymerase chain reaction using virus specific or degenerate primers. To amplify an informative region of the begomoviral coat protein of many begomoviruses, degenerate primers are available (Wyatt and Brown, 1996). These primers were updated in 2006 (Idris and Brown, unpublished). The former primers facilitate amplification and sequencing of an informative fragment of the coat protein gene in one forward and reverse reaction. If not confounded by recombination, the reactions permit tentative identification of begomoviral species without the requirement to sequence the entire genome. A non-sequence specific amplification and cloning of genome-length units, referred to as rolling circle amplification (RCA) is used to circumvent the lack of virus-specific primers to facilitate amplification of all known helper viral genomes, and the multitude of recombinants (Haible, et al., 2004; 2006; Inoue-Nagata et al., 2004). PCR primers that amplify the majority of, but not all, begomovirus-associated satellites are available (Amrao et al., 2010; Briddon et al., 2002; Bull et al., 2003; Idris et al., 2002; 2005; 2011). Polymerase chain reaction and RCA diagnostics should be implemented concurrently because RCA does not always detect satellite molecules that may be present (Brown, unpublished results).

Because it has been shown that the satellites are involved in virulence, symptom development, and resistance breaking, it is essential to accurately detect and identify the entire complex of helper virus and the associated satellites. Once achieved, the next important step is to identify the source of the introduction, and to understand the key biological features of the complex, in particular, the host range. Some leaf curl disease hosts may not show symptoms until late in infection or at all, particularly ornamentals or some wild hosts. Further, symptoms can be confused with other well-known endemic viruses and an introduction can be easily overlooked until the virus complex has spread and become established over large areas, in ornamental, vegetable, and fiber crops.

V. USDA Pathogen Permits and Regulations

USDA-APHIS-PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring Select Agents are required to be registered; however, diagnostic screening laboratories that identify select agents from a suspect sample are exempt from this requirement as long as an APHIS/CDC

Form 4 is completed, and the culture(s) are destroyed within 7 calendar days (Floyd, 2007).

The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status, that when shipped interstate require the receiving laboratory to have a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at <http://www.aphis.usda.gov/ppq/permits/> [accessed August 11, 2009] or contact PPQ Permit Services at 301-734-0841.

The Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies the requirements for possession, use, and transfer of organisms listed as Select Agents such as R3b2. Once an unregistered diagnostic laboratory identifies or suspects a Select Agent, they must immediately notify the APHIS Select Agent Program (within 24 hours of confirmation), complete an APHIS/CDC Form 4 and either destroy or transfer the agent to a registered laboratory within 7 days. In compliance with this Act, if a diagnostic laboratory held back part of a screened sample or culture for voucher purposes and that sample forwarded to the USDA Beltsville Laboratory came back as positive for a Select Agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. This must take place within 7 calendar days of results notification and a PPQ Officer must be provided with the opportunity to witness the destruction of the sample or culture within that time period. Clarification of this and other information related to adherence to the Select Agent regulations is available on the following APHIS website: http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml or contact the APHIS Select Agent Program 301-734-5960.

Researchers wishing to work with foreign plant pathogens in the U.S. should review the websites listed above and contact the PPQ permit unit to understand how best to comply with the permitting requirements.

VI. Response

The response to all plant health emergencies is under USDA-APHIS-Plant Protection under The Plant Protection Act of 2000 (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7CFR Part 331).

The planned immediate response to a begomoviral-like suspect would be to determine the identity of the causal agent(s), including the helper virus and associated satellites (alpha or beta complex). Although there are very few RNA viruses known to infect cotton, their presence should not be ruled out until additional information, including the prospective insect vector(s) on site (or observed consistently with the symptoms) are explored. Subsequent to ruling out other potential causes that present similar symptoms in the plants, immediate action should be taken to detect and determine the identity of the suspect viral complex associated with symptomatic plants, followed by completion of Koch's Postulates, in so far as this is possible (predominant viral helper and beta satellites, at the least) given the need to construct full-length infectious clones (see *Detection and Identification*, section IV).

After a confirmed detection by the USDA-APHIS-PPQ recognized authority, APHIS, in cooperation with the Department of Agriculture is in control of the response. The response is an immediate assessment consisting of investigation and delimitation of the site of initial detection to

prevent pathogen spread and to establish extent of the affected area. The team will also assess whether the introduction was intentional or accidental. As a plant pathogen on the select agent list, CLCuV is covered under the Agricultural Bioterrorism Protection Act of 2002; federal and local law enforcement may be involved to determine if a bioterrorism event has occurred.

APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant pathogens or regulated articles and works in conjunction with states to impose these actions parallel to state regulatory actions to restrict intrastate movement.

VII. Economic Impact and Compensation

Cotton and textile industries are central to the economic well being of developed and lesser-developed countries. Cotton production contributes heavily to food security in Africa, Asia, and Latin America. It is grown in 100 countries and occupies about 2% of the world's arable land. It is among the most significant crops after field grains and soybeans. Over the last three decades, the four leading cotton-producing countries have been China, India, the United States, and Pakistan accounted for about 75% of the world's production in 2010. Cotton production activities involve over 250 million individuals, and millions more in related industries, worldwide. Cotton is widely traded and over 150 countries are involved in import and/or export activities related to cotton. The industry produced 22 million tons of cotton worth approximately 37 billion U.S.dollars in 2010-2011. On average, the cost to produce cotton is about sixty cents (U.S.) per pound minus the cost of land rent and seed after ginning, excluded from the cost of (U.S. \$1.22/kg lint) (Cost of Production of Raw Cotton: Technical Information Section, International Cotton Advisory Committee, 2010; personal communication, R. Chaudhry, ICAC).

Yields of cotton have risen steadily over time from 230 kilograms/hectare (kg/ha) in the 1950's to 600 kg/ha in 1991-92. Since then yields have stagnated due to insect and disease problems, which have increased with increased intensity of production and the introduction of varieties having high yields and superior quality, while lacking sufficient pest and disease resistance. Varietal improvement through breeding programs and biotechnology made possible yields of 795 kg/ha in 2007-2008. The average rate of increase between 1950 to the present has been a total of 9kg/ha per year.

The widespread production of cotton in Asia and Africa since the initial outbreak and spread of, and the recent trade in ornamentals and vegetable seedlings between CLCuMV and CLCuBV infected and uninfected areas is cause for concern. The introduction and establishment of extant, as well as potential emergent, variants of the *Cotton leaf curl virus* complexes of Asia and Africa outside of their zone(s) of endemism could result in serious outbreaks. Because the whitefly vector is present in all cotton growing regions of the world, a single introduction followed by spread could rapidly overtake production areas where the virus is not endemic. Because all local varieties would likely be susceptible, extremely rapid spread, not only to cotton crops but also to certain susceptible vegetable crops and to ornamentals grown in landscapes in the rural-urban interface would be expected. The rapid spread into the U.S. cotton and vegetable crops alone would result in huge economic losses similar to those experienced with the Asian complexes in Pakistan and India. This is because our ornamental, vegetable, and cotton production areas in the United States and elsewhere in Latin America are tightly interconnected geographically. As a result, an introduction into one commodity can readily affect another particularly when it concerns the highly polyphagous whitefly vector and begomovirus complexes they transmit, both of which have broad and overlapping host ranges. Further, many ornamental hosts may harbor both the virus and the vector, making the

situation even more precarious because the whitefly vector can transmit the virus for life, once acquired, and so do not need to be transported on an infected plant to pose a threat when it comes into contact with a susceptible host. Not ornamentals or vegetable seedlings that are infected exhibit easily recognizable symptoms, particularly if they are shipped shortly after inoculation and before they have developed full-blown symptoms. A number of ornamental host species are suspected to be somewhat tolerant to the virus and can serve as symptomless carriers. Finally, the host range of the leaf curl virus complexes are poorly studied, and the most recent research underway in Pakistan and India is revealing that the host range of these viruses span far more genera and families of plant than previously known (Brown et al., unpublished results).

No information is available at this time regarding compensation.

VIII. Mitigation and Disease Management

Whitefly control

The recommendations for managing population size and density apply to thresholds established for management of the whitefly *B. tabaci* as a pest, not as a vector. The population threshold size per plant for *B. tabaci* as a vector is one, because a single viruliferous whitefly is capable of transmitting the virus, despite the overall reduction in whitefly population sizes in general (due to pesticide control). At different times of the year, the frequency of viruliferous whiteflies in a population is expected to vary, and to increase as the season progresses, owing to secondary virus infection rates in cotton and alternate hosts. Thus whitefly control alone, particularly in cotton-vegetable cropping systems, or when planting dates overlap, not allowing a sufficient host free period, will not result in quarantine significant control of the vector populations. This is particularly true during early growth stages of the crop when the primary virus inoculum levels are high. This latter situation occurs frequently in the crop in Pakistan owing to the prevalence of diverse viruses having broad host range.

Cultural control of whiteflies is possible only if the dates of planting and harvesting of all whitefly-susceptible crops can be synchronized over a broad area, and crop-free periods are established and adhered to. Because the *B. tabaci* biotypes associated with (adapted to) agricultural systems have extremely broad host ranges, and generally disperse moderate to long distances, cultural control of the whitefly is not very effective.

Insecticide control of whitefly to reduce virus spread

Insecticide programs in place vary depending on the cotton growing area of the United States. Specific references can be sought out for those areas through the Cooperative Extension Service and the U.S. Western Region Integrated Pest Management Center (<http://www.wrpmc.ucdavis.edu>). Selective insecticides, i.e. whitefly-specific versus broad spectrum, have the greatest success because they do not kill natural enemies, need fewer treatments per season, and tend to develop resistance more slowly. It is important not to mix broad spectrum and selective insecticides unless a mixture is required to manage a complex of insect problems in the crop. The choice of materials depends on the risks of economic loss, the potential for unmarketable lint due to honeydew contamination, and the risk of developing resistance to valuable non-chemical and chemical tactics that promote survival of predators and parasitoids, as well as the other natural fauna.

Insecticide resistance management is accomplished by limiting the number of treatments, using a diverse class of compounds and modes of action, and partitioning and sharing chemistries across crops. Refer to the three stage management plan for controlling *B. tabaci* at pest thresholds outlined in Ellsworth et al., (2006).

Protective measures

Reflective mulches and floating row covers deter and protect plants from whitefly infestations, respectively. This is relevant to cotton-vegetable mixed cropping systems because only certain kinds of vegetables in certain production settings are economically feasible. Row covers are effective only if plants are protected during early- and mid-growth stages because once plants become infected with begomoviruses, growth is arrested and fruit is harvestable primarily from the portion of the plant in production at the time of removal. Row covers are expensive and not practical for cotton production and large-scale vegetable production. Greenhouses and screen houses (e.g., for ornamentals or controlled environment vegetable production such as tomatoes and peppers) can be protected by outfitting the structures with fine mesh screens to protect against whitefly infestation and therefore reduced inoculation that results in transmission of virus to plants by dispersing, viruliferous whiteflies. This is not practical for field crops.

Resistance to the Cotton leaf curl complex and other begomoviruses of cotton

Disease resistance is the only effective way of managing leaf curl disease, particularly when infection occurs early and routinely in the production season. The variability in the natural incidence of disease depends upon the genetic makeup of the cultivar, concentration of inoculum of the disease and cultural management at different sites. Further, the pressure of whitefly with concurrent presence of inoculum in the area influences disease incidence (Baluch, 2007; Tahir and Mahmood, 2005).

Conventional selection and breeding approaches that involved transferring resistance genes from wild species to Upland cotton eventually yielded varieties with excellent resistance to CLCuMV even though early screening and selection trials revealed a wide range in degrees of resistance across the offspring (Ahuja et al., 2007; Baluch, 2007; Naveed and Anjum, 2007; Tahir and Mahmood, 2005; Tahir et al., 2005). The widespread cultivation of the resistant varieties throughout Pakistan (except in Sindh) subsequently resulted in highly effective management of CLCuD. A genetic study of the offspring of crosses and selections from the parental donors of the resistance genes, LRA5166, CP-15/2, and Cedix, indicated that three genes conferred resistance. Two of the genes that contributed to virus resistance and a further gene imparted suppression of the symptom development (Tahir and Mahmood, 2005).

Currently, sources of genetic resistance are lacking to manage the recently emergent, recombinant CLCuBV that predominates in Pakistan and northwest India. Breeding efforts are again underway in the region to screen a wide array of germplasm to identify sources of resistance genes. It has been reported that the genetic stock maintained by different research institutions in Pakistan has a narrow genetic base, and that all are highly susceptible (Mahmood, 1999; Mahmood et al., 2002; Tahir and Mahmood, 2005). Therefore, the introduction of exotic materials could possibly aid in widening the genetic base for inclusion of resistance in Upland cottons. The variability in reaction of the different cotton stock is dependent upon its genetic makeup and environmental conditions, including the extent of whitefly vector and therefore virus pressure.

Diploid cotton (*G. arboreum* and *G. herbaceum*) grown across Asia and Africa prior to the introduction of tetraploid cottons (*G. hirsutum* and *G. barbadense*) is immune to CLCuD (Mahmood, 1999). A recent study on cotton species grown in the 'living herbarium' maintained at CCRI Multan has identified other sources of resistance in wild species of cotton (Azhar et al., 2010). The major obstacles are ploidy barriers that require several steps to introduce characters from diploid to tetraploid cotton. This is problematic due to the lack of understanding of resistance mechanisms in diploid Asiatic species, and the absence of DNA markers linked to disease resistance. Other strategies have involved the incorporation of traits from *G. hirsutum* into *G. arboreum* ('hirsutization' of *G. arboreum*), and cloning genes from *G. arboreum* for transgenic introgression. Even so, additional efforts are required to determine the durability of the varieties produced using either of these strategies.

Several promising lines have been identified with tolerance or resistance to CLCrV in the United States (Wilson and Brown, 1991). Resistance has not been incorporated into varieties grown there because infection occurs mid to late season in most years. In years when whitefly population levels increase earlier than usual, inoculation of cotton by viruliferous whiteflies occurs when cotton is in the early growth stages and susceptible to significant damage (Brown et al., 1997; Butler et al., 1986; van Schaik et al., 1962).

Transgenic resistance to begomovirus infection

A number of research efforts have been initiated and/or are underway to explore the use of virus-derived resistance (Beachy, 1997) to manage the leaf curl complex in cotton and/or in alternate hosts such as tobacco or tomato (Brown et al., unpublished; in progress). Sense, anti-sense, and more recently small RNA-interference approaches have been investigated. Various viral-encoded genes as well as the beta-satellite C1 ORF have been considered as targets, as well as combinations of multiple targets yielded some promising results when tested in easy to transform species such as tobacco (Asad et al., 2003; Zafar and Brown, 2011; Zafar et al., 2003).

The high genetic diversity in the extant viral species in Pakistan and India and the ability of begomoviruses and beta satellites to occur in mixed infections and undergo recombination warrants careful scrutiny to select regions of high homology across the entire complex to avoid selection of a highly virulent recombinant. The use of small RNAi strategies (Hamilton and Baulcombe, 1999), that although highly sequence dependent, require only short targets to be effective. Another important constraint has been the inability to reproduce authentic leaf curl symptoms in cotton when inoculated with the cloned components of CLCuMV helper virus- betasatellite complexes (Briddon et al., 2000), although in surrogate tobacco plants, the clones are shown to be infectious, producing leaf curl symptoms following inoculation of the plants (Briddon et al., 2001). The subsequent recovery of the viral helper genome and betasatellite DNAs from symptomatic plants has demonstrated that at least in tobacco the clones have activity that are expected to be transferable to cotton likewise inoculated with them.

IX. Current Infrastructure, Needs and Experts

Little if any infrastructure is in place in the United States other than several laboratories specializing in begomovirus diseases of cotton (J.K. Brown). Several U.S. virology labs specializing in geminiviruses exist that could assist with emergency detection if needed; but no organized protocols or action plan is in place.

A number of entomologists (AZ, CA, FL, TX) are experts in whitefly management, and insecticide resistance.

See: The University of Arizona Cooperative Extension IPM Series No. 18. AZ1404-5/2006.
<http://cals.arizona.edu/pubs/insects/az1404.pdf>

X. Research, Education and Extension: Relating to whitefly management

<http://cals.arizona.edu/crops/cotton/cotton.html>
<http://www.ipm.ucdavis.edu/PMG/r114300311.html>
<http://ag.arizona.edu/crop/cotton/insects/wf/ipm6.html>
http://www.icac.org/projects/commonfund/ipmc/proj_02_final.pdf

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